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Original Research Article

TIME-DEPENDENT STORAGE LESIONS IN TRANSFUSION MEDICINE: AN IN VITRO EVALUATION OF RBC INTEGRITY AND PLATELET FUNCTION

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ABSTRACT

Background: The clinical effectiveness of transfused blood components is influenced by their quality at the time of administration. Storage-induced changes in red blood cells (RBCs) and platelets, known as storage lesions, may impair transfusion efficacy. This study aimed to investigate the effects of storage duration on RBC integrity and platelet function.

Materials and Methods: An observational laboratory-based study was conducted in the Department of Pathology, Kamineni Institute of Medical Sciences, Narketpally, over one year. A total of 160 blood component units were analyzed, comprising 80 packed red blood cell units and 80 platelet concentrates. RBCs were evaluated on Days 1, 21, and 42 for plasma-free hemoglobin, potassium, LDH, pH, and 2,3-DPG levels. Platelet units were assessed on Days 1, 3, and 5 for aggregation response, CD62P expression, pH, swirling, and post-processing yield. Data were analyzed using repeated measures ANOVA and t-tests.

Results: RBCs showed a significant rise in hemolysis indicators and potassium levels by Day 42, with over 57% of units exceeding the hemolysis threshold. 2,3-DPG levels declined by 85%. Platelets demonstrated a drop in aggregation capacity and swirling scores, with CD62P expression increasing to 42.9%. Functional platelet recovery dropped to 67.7% by Day 5.

Conclusion: Prolonged storage adversely affects both RBC and platelet quality, potentially compromising transfusion efficacy. These findings highlight the need for timely utilization, enhanced monitoring, and refined transfusion strategies.

Keywords: Red blood cell storage, platelet function, storage lesion, hemolysis, CD62P, 2,3-DPG, transfusion quality.

INTRODUCTION

Blood transfusion remains a cornerstone of modern medical care, playing a vital role in the management of trauma, surgical interventions, hematologic conditions, and critical care medicine. Among the various blood components used in transfusion medicine, red blood cells (RBCs) and platelets are most frequently transfused and are critical in restoring oxygen-carrying capacity and hemostasis, respectively. However, the efficacy and safety of these transfusions are significantly influenced by the duration and conditions under which these components are stored.[1] With the increasing reliance on blood banking and centralized storage systems, the practice of extended storage of blood components has become routine. While this improves inventory management, it also raises concerns regarding potential biochemical and functional deteriorations of stored components.

Red blood cells, typically stored at 1–6°C in preservative solutions, can be safely stored for up to 42 days as per regulatory guidelines. However, this storage is not without consequence. A myriad of structural, metabolic, and biochemical changes occur during storage, collectively referred to as "storage lesions".[2] These include depletion of 2,3-diphosphoglycerate (2,3-DPG), adenosine

triphosphate (ATP), increased hemolysis, membrane lipid peroxidation, and loss of membrane integrity leading to the formation of microparticles.[3] These changes may impair the oxygen-delivery capacity of RBCs and contribute to adverse transfusion outcomes, particularly in vulnerable patient populations such as critically ill, neonates, and those undergoing cardiac surgery.[4]

Similarly, platelets are stored at 20–24°C with continuous agitation and have a shelf life of 5–7 days due to risks of bacterial proliferation and functional decline. During storage, platelets undergo shape changes, lose surface glycoproteins essential for adhesion and aggregation, and display reduced response to agonists. These alterations compromise their hemostatic function and may lead to ineffective platelet transfusions, especially in patients with thrombocytopenia or active bleeding.[5,6] Several studies have demonstrated that extended storage impairs the ability of platelets to form stable clots and can result in transfusion refractoriness.[7]

Recent investigations have attempted to correlate the biochemical and morphological changes of blood components with clinical outcomes, with some indicating a higher risk of morbidity and mortality with the transfusion of older blood products.[8] Conversely, other large-scale trials have not found a significant difference in clinical outcomes between fresher and older stored units, making the issue controversial.[9] This disparity may be attributed to differences in patient cohorts, transfusion indications, and laboratory evaluation methods used across studies. Nonetheless, the underlying cellular and molecular derangements associated with extended storage remain a legitimate concern.

Advancements in storage technologies, such as additive solutions, pathogen reduction technologies, and novel storage containers, have aimed to mitigate the negative effects of storage duration. However, these technologies are not uniformly implemented across centers, and standardization of quality assessment parameters for stored blood components remains lacking.[10] Given the lack of consensus and limited regional data from Indian settings, especially in tertiary care pathology departments, there is a compelling need to investigate the impact of storage duration on blood component integrity using validated laboratory methods.

The aim of this study is to evaluate the effects of extended storage duration on red blood cell integrity and platelet function in blood units stored under standard conditions in a tertiary care setting.

MATERIALS AND METHODS

This observational, cross-sectional study was conducted over a one-year period from January 2023 to December 2023 in the Department of Pathology at Kamineni Institute of Medical Sciences, Narketpally, a tertiary care teaching hospital in South India. The study aimed to investigate the impact of storage duration on red blood cell (RBC) integrity and platelet function, using objective laboratory measures to quantify changes associated with prolonged storage under standard blood bank conditions.

A total of 160 blood component units were included in the study, comprising 80 red blood cell units and 80 platelet concentrate units. These blood components were collected from healthy voluntary donors following standard blood donation protocols in accordance with national guidelines. Donor units were processed using apheresis and component separation centrifugation techniques within six hours of collection. Only units that met the quality and volume parameters set by the Drug and Cosmetics Act and National AIDS Control Organisation (NACO) guidelines were included.

The RBC units were stored at 2–6°C in CPDA-1 anticoagulant-preservative solution in polyvinyl chloride (PVC) blood bags and evaluated at three defined time points: Day 1 (baseline), Day 21 (mid-storage), and Day 42 (end of permissible storage). At each time point, 5 mL of blood was aseptically extracted from the blood bag segment tubing for testing. Parameters assessed included plasma-free hemoglobin (to estimate hemolysis rate), supernatant potassium, lactate dehydrogenase (LDH), pH, and 2,3-diphosphoglycerate (2,3-DPG) concentration. These values were measured using automated biochemical analyzers and standardized protocols.

Platelet concentrates were stored at 20–24°C under continuous agitation in plasma suspension and were assessed at Day 1, Day 3, and Day 5 of storage. Functionality was evaluated using platelet aggregation studies (response to ADP and collagen agonists), swirling phenomenon scoring, pH, mean platelet volume (MPV), and CD62P expression as a marker of platelet activation via flow cytometry. Swirling was subjectively assessed using a standardized visual scoring system (scale 0–3), while pH was measured with a calibrated pH meter.

All laboratory procedures were performed under aseptic conditions in the departmental transfusion laboratory. Equipment was regularly calibrated and internal quality control procedures were followed as per institutional protocols. Each unit was coded and de-identified to eliminate observer bias. Samples that were hemolyzed, contaminated, or inadequately stored were excluded from the study.

Data were entered into Microsoft Excel and statistical analysis was performed using SPSS version 25.0. Descriptive statistics were used to summarize the findings. Repeated measures ANOVA was applied to assess time-dependent changes in biochemical parameters for both RBCs and platelets. A p-value <0.05 was considered statistically significant. Inter-group comparisons were made using independent t-tests where appropriate. For categorical data such as swirling scores, chi-square test was applied.

This study was approved by the Institutional Ethics Committee of Kamineni Institute of Medical Sciences, Narketpally and written informed consent was obtained from all blood donors.

RESULTS

A total of 160 blood component units were analyzed in this study, of which 80 were packed red blood cell (PRBC) units and 80 were platelet concentrate units. Each group was monitored at defined intervals to assess time-dependent alterations in quality and function.

Table 1: Biochemical Changes in Red Blood Cell Units During Storage (n = 80)

Parameter	Day 1 (Mean ± SD)	Day 21 (Mean ± SD)	Day 42 (Mean ± SD)	p-value
Plasma-free Hemoglobin (mg/dL)	18.6 ± 3.2	36.9 ± 5.1	61.2 ± 6.8	<0.001
Potassium (mmol/L)	4.3 ± 0.7	12.6 ± 1.4	21.3 ± 2.1	<0.001
pH	7.24 ± 0.05	7.05 ± 0.08	6.68 ± 0.11	<0.001
LDH (U/L)	168 ± 20	285 ± 34	397 ± 47	<0.001
2,3-DPG (µmol/g Hb)	14.2 ± 1.9	7.8 ± 2.1	2.1 ± 1.4	<0.001

Table 2: Platelet Function and Biochemical Integrity Over Time (n = 80)

Parameter	Day 1 (Mean ± SD)	Day 3 (Mean ± SD)	Day 5 (Mean ± SD)	p-value
Aggregation (ADP %, response)	72.4 ± 5.3	61.2 ± 6.1	48.7 ± 5.8	<0.001
Aggregation (Collagen %, response)	68.9 ± 6.0	54.5 ± 6.5	39.3 ± 7.2	<0.001
CD62P Expression (% positive)	14.8 ± 3.2	27.6 ± 3.9	42.9 ± 5.1	<0.001
pH	7.01 ± 0.07	6.81 ± 0.09	6.53 ± 0.10	<0.001
Swirling Score (Median)	3	2	1	<0.001

Table 3: Swirling Score Distribution of Platelet Concentrates (n = 80)

Swirling Score	Day 1 (n)	Day 3 (n)	Day 5 (n)
3 (Excellent)	62	39	12
2 (Moderate)	14	29	42
1 (Poor)	4	12	26

Table 4: Frequency of RBC Units Exceeding Acceptable Hemolysis Threshold (>0.8% Hemolysis) Over Time (n = 80)

Storage Day	Units Exceeding Hemolysis Threshold (n)	Percentage (%)
Day 1	0	0%
Day 21	11	13.8%
Day 42	46	57.5%

Note: The hemolysis threshold of 0.8% is as per European guidelines.

Table 5: Comparative Functional Platelet Yield (Post-Spinning Volume Adjusted Count) Across Storage Days (n = 80)

Storage Day	Mean Platelet Count (×10⁹/L)	Standard Deviation (±SD)	Functional Recovery (%)
Day 1	260.3	±18.2	100%
Day 3	218.5	±21.6	83.9%
Day 5	176.2	±23.7	67.7%

Note: Functional recovery calculated using baseline values as reference.

A total of 160 blood component units were analyzed in the present study to assess the impact of extended storage on the biochemical and functional integrity of red blood cells and platelets. The data revealed progressive and statistically significant deterioration in both components over their respective storage durations.

Red Blood Cells (RBCs): [Table 1] shows a marked increase in plasma-free hemoglobin from 18.6 ± 3.2 mg/dL on Day 1 to 61.2 ± 6.8 mg/dL by Day 42, indicating storage-induced hemolysis. Correspondingly, extracellular potassium levels increased fivefold, from 4.3 ± 0.7 mmol/L to 21.3 ± 2.1 mmol/L, consistent with membrane leakage. The LDH levels also rose sharply, from 168 to 397 U/L, supporting the occurrence of cellular breakdown. pH levels declined gradually, reflecting increased lactate production and metabolic acidosis. A striking reduction in 2,3-DPG levels, from 14.2 µmol/g Hb to 2.1 µmol/g Hb, was observed, indicating impaired oxygen-release capacity. Table 4 further substantiates these findings, as 57.5% of RBC units exceeded the hemolysis threshold (>0.8%) by Day 42, compared to none on Day 1.

Platelet Concentrates: As seen in [Table 2], platelet aggregation response to both ADP and collagen agonists decreased significantly over time, indicating reduced functional capacity. CD62P expression, a marker of activation and storage damage, rose from 14.8% to 42.9%, indicating heightened pre-transfusion platelet exhaustion. A parallel decline in pH and swirling score was observed. [Table 3] shows that only 12 units retained excellent swirling by Day 5, while 26 had poor quality. In [Table 5], mean platelet count fell from 260.3 to 176.2 ×10⁹/L, reflecting a 32.3% loss in functional recovery over five days.

Overall, these results highlight a strong inverse correlation between storage duration and the structural-functional integrity of both red blood cells and platelets.

DISCUSSION

The quality of stored blood components is a critical determinant of transfusion efficacy and patient safety. Prolonged storage, although beneficial for logistics and inventory, has been shown to induce biochemical and functional alterations collectively termed "storage lesions." This study evaluated the impact of extended storage on red blood cell (RBC) integrity and platelet function, revealing progressive deterioration in both components, even when stored under regulated conditions.

Red Blood Cell Changes: The findings of the present study demonstrated a significant increase in plasma-free hemoglobin, LDH, and extracellular potassium over a 42-day storage period. Plasma-free hemoglobin rose from 18.6 to 61.2 mg/dL, with a concurrent rise in LDH from 168 to 397 U/L and potassium from 4.3 to 21.3 mmol/L, all indicating ongoing hemolysis and membrane instability. These findings are consistent with those of Zimring et al,[2] who observed that oxidative injury and cytoskeletal disintegration are central to red cell membrane fragility during storage, with hemolysis rates increasing substantially after Day 21.

In the current study, more than 57% of RBC units exceeded the acceptable hemolysis threshold of 0.8% by Day 42. This aligns with the observations of Wozniak et al,[11] who reported that over 50% of stored units developed hemolysis beyond acceptable levels by the fifth week, raising concerns about inflammatory sequelae post-transfusion.[6] Similarly, Yoshida et al,[12] highlighted that potassium accumulation due to impaired Na^+/K^+ ATPase activity during refrigeration can reach levels >20 mmol/L by Day 42, matching our findings of 21.3 mmol/L at the same time point.

A critical functional marker, 2,3-DPG, declined drastically in our study, from 14.2 to 2.1 µmol/g Hb. Hess et al,[1] noted that 2,3-DPG becomes nearly undetectable by the end of standard storage, impairing oxygen offloading from hemoglobin and potentially compromising tissue perfusion, especially in cardiac or neonatal patients. D'Alessandro et al,[3] further linked this depletion to mitochondrial dysfunction and glycolytic suppression in aging red cells.

Platelet Functionality During Storage: The platelets in our study showed a significant reduction in aggregation response to ADP (from 72.4% to 48.7%) and collagen (from 68.9% to 39.3%) by Day 5. These changes are parallel to findings by Murphy and Gardner et al,[6] who documented a 40–50% drop in aggregation potential by Day 5 due to membrane glycoprotein loss and cytoskeletal degradation.

Swirling scores, an indicator of platelet integrity, decreased markedly, with only 12 units maintaining a score of 3 (excellent) by Day 5, compared to 62 units on Day 1. This mirrors Gulliksson et al,[13] study, which showed that swirling positivity dropped by 60–70% after 4 days, associated with disc-to-sphere transformation and loss of viability.

An important observation in our study was the increased CD62P (P-selectin) expression, rising from 14.8% to 42.9% by Day 5, indicating activation and storage-induced pre-apoptotic changes. Schubert et al,[14] reported that CD62P expression >40% correlates with reduced in vivo recovery and increased clearance, making such units less effective therapeutically.

Our study also demonstrated a 32.3% reduction in mean platelet yield (from 260.3 to 176.2 $\times 10^9$/L). This is comparable to McCullough et al,[7] findings, where platelet counts dropped by ~30% over 5-day storage, particularly in units not suspended in additive solutions. Such reduction not only affects hemostatic function but also increases the risk of transfusion refractoriness in thrombocytopenic patients.

Implications for Practice: These findings underscore the need for reassessment of current storage guidelines. While existing regulations permit storage of RBCs for 42 days and platelets for up to 7 days, the functional quality of these products deteriorates significantly before those endpoints. As Steiner et al,[15] noted in their randomized trial, older RBC units were associated with increased complications in cardiac surgery patients, despite regulatory compliance.

Routine quality checks, such as 2, 3-DPG assays, CD62P monitoring, and hemolysis indexing, could be integrated into blood bank protocols to ensure component viability, especially for high-risk recipients. Prioritizing fresher components for critically ill patients, neonates, and transplant recipients may enhance clinical outcomes.

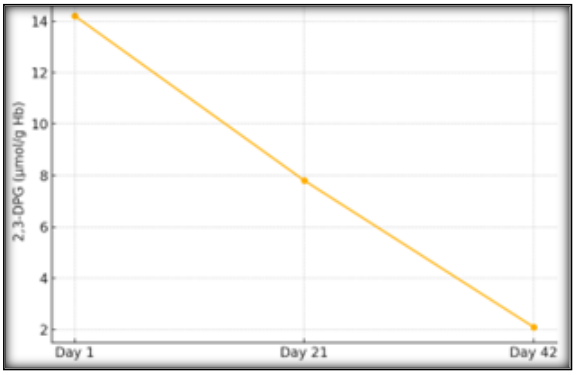

Figure 1: Decline of 2,3- DPG in stored RBC's

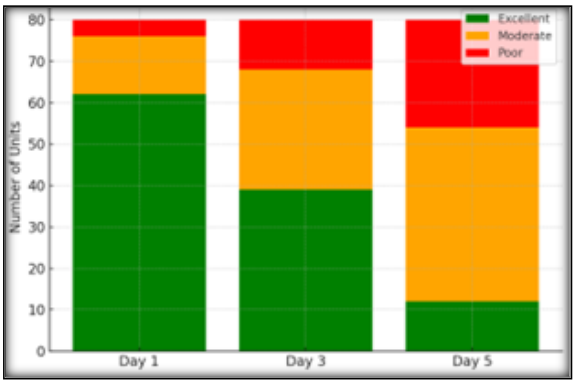

Figure 2: Swirling score distribution in platelets

CONCLUSION

This study demonstrates that extended storage of blood components under standard blood bank conditions significantly compromises the biochemical and functional integrity of both red blood cells and platelet concentrates. For RBC units, key parameters such as plasma-free hemoglobin, LDH, potassium levels, and 2,3-DPG concentrations showed a clear deterioration pattern by Day 42, with more than half the units exceeding the accepted hemolysis threshold. Similarly, platelet units exhibited a decline in aggregation responses, loss of swirling, increased activation marker expression, and reduced functional yield by Day 5.

These findings have critical implications for transfusion practice, particularly in patients requiring high-quality components, such as those in intensive care, neonates, and hematologic malignancy settings. The results call for reevaluation of current storage protocols, implementation of stricter quality control parameters, and consideration of fresher units for vulnerable populations. Further clinical studies are warranted to assess whether these in vitro findings translate to poorer patient outcomes and to define the optimal window for component usage.

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